SMEAR AND CULTURE DIAGNOSIS IN GONORRHŒA*

BY

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The subject of the diagnosis of gonorrhœa may be considered under three main headings apart from clinical diagnosis. The first is how far smear diagnosis is reliable and what are its limitations; the second is whether culture methods are superior to or at least adjuvant to direct microscopical observations and, if so, what the best methods may be; and the last to what extent reactions based on immunity supplement the above methods.

Diagnosis from Direct Microscopical Examination of Smear Preparations

Everyone is familiar with the appearance of a typical smear from acute gonorrhœa containing numerous intra-leucocytic diplococci. In more chronic and subacute infections, however, there may be preparations in which the gonococci are not easily recognized, and this is particularly so in those from females, in whom other bacteria are often present, and in whom the numbers of gonococci tend to fluctuate. An American worker has recently stated that the superiority of culture over smear methods has been overstated. I cannot agree, and I want to make one or two points which force themselves on any bacteriologist who does much of this kind of work.

When there are only small numbers of gonococci in a preparation it becomes difficult to identify them with certainty, and there are two reasons for this. First, Gram-positive cocci present in small numbers will on occasions have been taken up by the phagocytes and will have lost their Gram-staining. It is difficult to differentiate between staphylococcal and other Gram-positive cocci which have lost their usual staining on the one hand, and scanty gonococci on the other. Secondly, there are undoubtedly a minority of Gram-negative cocci which appear in the genital tract which are not gonococci; that is my experience, and also the experience of other observers, for example Reymann (1941, 1943) in Scandinavia. Such organisms appear in 1 to 2 per cent. of cases.

Table I shows the characteristics of six strains of Neisseria which were obviously not gonococci and which were obtained in a series of three hundred cases examined for gonorrhœa by culture. In one of these the colonies were quite numerous, and this was a case in which there was a positive smear. Here it is possible that examination of the smear alone might have led to a wrong diagnosis. In four other cases there were only a few colonies of Gram-negative diplococci, but as the smear was negative a mistake was unlikely to have arisen. However, as long as such possibilities exist, even in a few cases, it would be difficult to appear in a Court of Justice and plead with conviction that a

TABLE I

GRAM-NEGATIVE DIPLOCOCCI, NOT GONOCOCCI, FOUND IN COURSE OF INVESTIGATION OF 300 CASES

Culture	Sugars	Agar	Smear
1. Opaque pigmented colonies ++	0	0	+
2. One colony, convex and adherent	G+ M+ S+	0	−
3. Colonies ++ convex, opaque	G+ M+ S−	+	−
4. Colonies scanty, opaque, rather yellow	0	+	−
5. Colonies scanty	G− M− S−	0	+
6. Diplococci tend to grow in chains	G+ M− S−	++	−

0=no observation

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typical preparation in which Gram-negative diplococci appeared necessarily showed the presence of gonorrhœa.

Methods of Culture

Table II shows smear and culture results in a series of 275 cases under the care of Dr. Dorothy P. Priestly, who was in charge of the female venereal disease clinic at Leeds. They were suspected cases of gonorrhœa, and, according to the combined smear and culture results, 50 per cent. were positive. Smears were described as "typical" which showed numerous Gram-negative intracellular diplococci. "Completely identified" under culture means that the organism was separated in pure culture, that the sugar fermentations were correct, and that inability to grow on agar was demonstrated. The remainder were identified on the grounds that typical colonies which gave a positive oxidase reaction were shown to be made up of Gram-negative diplococci.

TABLE II

SMEAR AND CULTURE RESULTS COMPARED (275 CASES)

Smear	Culture
29% positive (20% typical)	42% positive (29% completely identified)
Both C. and U. positive in 7%	Both C. and U. positive in 22%
Urethra positive in 24%	Urethra positive in 32%
Cervix positive in 16%	Cervix positive in 33%

50% of all cases positive by smear or culture

Table III demonstrates the possibility of improving the methods of culture for diagnosis. In 1927 we were simply doing culture on heated blood agar described as "chocolate." The numbers are too small to justify any firm conclusions, but the indication is of a rising percentage of positive results with improvement of the medium and the introduction and modification of the oxidase reaction. These earlier successes were followed up by recording the results in a big series of clinical cases. In a total of over 2,000 cases we obtained more than 100 per cent. increase in the positive findings by adding the culture results to those obtained by smear examination.

Some Theoretical Aspects

Table IV shows the results of a number of experiments undertaken some years ago in order to see how the gonococcus survived in various fluids which were not quite sufficient to maintain life. It would seem that meat extract alone supports the gonococcus poorly, and that peptone, though helpful, is not tolerated well when concentrated. These results suggest that there may be substances in peptone which are actually lethal to the gonococcus; and Table V indicates that blood added to the medium may protect from such substances. With a small amount of peptone, growth occurred with the addition of 1 per cent. of blood, but the initiation of growth became more difficult as the peptone was increased, until, with 5 per cent. peptone, growth was abundant only where 5 per cent. or more of blood was also present.

It was found that some amino-acids inhibited the growth of the gonococcus and others favoured it, as shown in Table VI. Partly as a result of that work, and partly as a result of experiment, we have finally adopted,

TABLE III

RESULTS OF CULTURES IN SUCCESSIVE PERIODS

Cases	Period	% Positive	Method
23	1927	17%	Chocolate
125	1927–28	31%	„ and dimethyl*
125	1928–29	50%	„ and tetramethyl*
25	Dec. 1929	56%	Special medium and tetramethyl

* Dimethyl, etc., means the use of a solution of dimethyl *p*-phenylene diamine hydrochloride as an indicator of oxidase + colonies.

TABLE IV

SURVIVAL OF GONOCOCCUS IN VARIOUS FLUIDS INADEQUATE TO MAINTAIN GROWTH

Medium	Time of survival (in minutes)
Meat extract +0·1% peptone	20
„ „ +0·25% „	160
„ „ +0·5% „	100
„ „ +1·0% „	60
„ „ +2·5% „	20
Water	25
Physiological saline ..	35
Ringer's solution	75

and for many years used the ordinary nutrient agar reinforced with blood; and as far as the gonococcus is concerned, heated blood (chocolate medium) seems to give better results than does unheated blood. There is also increased growth with an increased amount of blood, and for the gonococcus we use a medium of this kind and raise the blood concentration from the usual 5 or 10 per cent. to about 20 per cent.

Table VII shows the contrasts between *p*-phenylene diamine and its various methyl substitution products in respect of their reactions with mild and strong oxidizing agents on the one hand, and bacteria on the other. These compounds become more susceptible to oxidation as more methyl groups are introduced. The original unsubstituted product is difficult to oxidize; with the monomethyl compound a yellow-brown colour results, with the dimethyl compound a maroon colour, and with the tetramethyl compound a violet colour. With a strong oxidizing agent a black pigment is finally obtained with the first three, and the bacterial action corresponds to that of the strong oxidizing agent. With the tetramethyl compound there is a deep violet colour only, which probably means that the blackening depends upon some polymerization which is blocked by full methyl substitution. That is the theory of this reaction, which is by no means confined to the gonococcus:

TABLE V

TO SHOW THAT PEPTONE INTERFERENCE WITH GROWTH IS ELIMINATED AS AMOUNT OF HEATED BLOOD IN THE MEDIUM IS INCREASED

Blood concentration	Growth of gonococcus					
10%	++	++	++	++	++	++
5%	++	++	++	++	++	++
2%	++	++	++	++	+	+
1%	++	+	+	+	+	0
	0·1%	0·2%	0·5%	1%	2%	5%

Concentrations of peptone added to meat extract

TABLE VI

EFFECT ON THE GROWTH OF THREE STRAINS OF GONOCOCCUS OF AMINO-ACIDS IN BROTH ENRICHED WITH 0·25% BLOOD AND HEATED AT 75° C.

Amino-acid added	Strain 1	Strain 2	Strain 3
Glycine 0·5%	0	0	0
„ 0·1%	55 colonies	++	0
Taurine 0·5%	++	++	25 colonies
„ 0·1%	++	++	0
None—control	0	++	0
None—control	++	++	0

TABLE VII

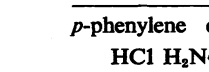
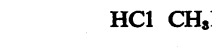
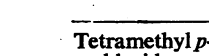
Reagent	Effect of mild oxidants, e.g., H_2O_2	Effect of strong oxidants, e.g., $K_2Cr_2O_7$	Effect of oxidase-active bacteria exposed to reagent and air
p-phenylene diamine hydrochloride 	—	Formation of black pigment	Slow development of black colour
Monomethyl *p*-phenylene diamine hydrochloride 	Faint yellowish-brown	Formation of black pigment	Rapid development of black colour
Dimethyl *p*-phenylene diamine hydrochloride 	Formation of maroon colour (Wurster's red)	Formation of black pigment	Slower development of black colour after preliminary maroon stage
Tetramethyl *p*-phenylene diamine hydrochloride	Formation of violet colour (Wurster's violet)	Formation of deep violet or purple colour	Development of deep purple colour after preliminary violet stage

TABLE VIII

THE MORE IMPORTANT OXIDASE POSITIVE AND NEGATIVE BACTERIA

Positive	Negative
Neisseria (strong)	Coliform group
Many vibrios (strong)	Salmonellæ
Brucellæ (moderate)	Corynebacteriæ
Hæmophilus (weak)	Streptococci
Anthrax and anthracoids (moderate)	Staphylococci
B. Mallei (moderate)	Sarcinæ
B. Pyocyaneus (moderate)	Anærobes

TABLE IX

FIGURES FOR SMEARS AND CULTURES FROM THE LEEDS BACTERIOLOGICAL LABORATORY

	Smears		Cultures	
	Number	%+	Number	%+
1938 : male ..	944	19·8	660	2·0
female ..	1,282	7·9	1,263	21·2
1944 : male ..	1,302	17·0	448	4·25
female ..	2,021	9·75	1,171	18·0
1946 : male* ..	—	—	1,653	3·5
female ..	5,530	4·0	5,642	10·0

* No smears from males examined in Bacteriological Department.

although there are many bacteria which are negative when tested in this way (see Table VIII). The great strength of the reaction is that no matter how a colony is overgrown it will show up. In this respect the results are superior to those obtained with media on which the pathogenic organism is colourless and the non-pathogenic is coloured, as on many media used for separating pathogenic coliforms from the fæces.

I do not want to claim for my colleagues and myself any originality in using the oxidase reaction, because it has been long under investigation by many observers. The earlier observations deal with questions like the oxidizing granules in the polymorphonuclear leucocytes and marrow cells, and its first use was mainly related to pathology. Christiansen and Becker (1938) claimed that the credit for introducing the oxidase reaction to bacteriology should go to Loele. Actually Loele drew attention to the possibilities of the oxidase reaction in a paper which appeared in 1928, the same year as one by Gordon and myself; and he extended these observations in 1929. His work should not have been overlooked in our subsequent publications (Ellingworth and others, 1929; McLeod and others, 1934). I note, however, from Loele's paper, that Würster drew attention to the oxidation of tetra-methyl *p*-phenylene diamine by yeast the year before I was born! Nevertheless the method does not appear to have been seriously used in the cultural diagnosis of gonorrhœa in Germany. No general practical application was in fact made until 1934, when we (Mcleod and others) published a paper on the subject of its use in the diagnosis of gonorrhœa. From that time it was extensively explored in America (Leahy and Carpenter, 1936; Thompson, 1937), and I am convinced that if those who have the responsibility of making bacteriological investigations for the detection of carriers of cholera were to use it, it would yield valuable results. There is no opportunity, however, of making practical demonstrations of that kind in this country, and I do not know that anyone has taken it up in countries where cholera is endemic.

In practice the oxidase method is most simply used by pouring a weak solution of tetra-methyl *p*-phenylene diamine hydrochloride over the plate and letting it run off, soaking away the excess with a piece of sterile blotting paper, and as soon as the colonies begin to dry picking off purple colonies of characteristic appearance. Although this reagent oxidizes spontaneously in air, it only develops a light violet colour, which deepens markedly in contact with the gonococcal colonies. The dimethyl compound, although also useful, has the disadvantage that the red pigmentation goes over to black and that at some stage in the production of the black colour there is a marked bactericidal effect.

Effect of Gaseous Environment on Growth of Gonococcus

In view of the action of gaseous environment on bacteria, and especially the influence of carbon dioxide on *Brucella Abortus*, the effect of gaseous environment was studied. On trying out the gonococcus in an atmosphere with increased CO_2 content, we got the impression that the results were better with some strains. This has been confirmed by both Danish and American observers (Reymann, 1941; Thompson, 1937), and our practice is to give cultures a preliminary incubation in a jar which has been flushed from a cylinder containing compressed air with 8 per cent. carbon dioxide.

Recent Results

Reymann published (in Danish: 1941; and in English: 1943) an extensive thesis on this subject. He found that by the methods described above (with slight modification) he was able to get better results than with the German methods which have been largely used and most often recommended (Neumann, 1938).

For the purpose of checking the value of these methods after they had been in routine use for a number of years and were not receiving quite the same close attention as in the initial period of trial and establishment, I took the figures from the books of the Leeds bacteriological laboratory for three representative years. The year 1938 represents a pre-war year, and 1944 a war year, during which the late Dr. Bibby was in charge of the Leeds Venereal Disease Clinic. The year 1946 is from the post-war period, when Dr. Lees was in charge.

The male smear and culture results do not cover the same cases. Smear examinations were used both for diagnosis and to check cure after treatment —cultures only for the latter. The female smear and culture results cover the same group of cases; and in them examinations for diagnosis were more frequent. Under the control of Dr. Lees and Dr. Walker the total number of examinations of female cases has been considerably greater and the percentage of positive reactions has fallen (Table IX). There has been more than 100 per cent. increase over smear results in positive culture examinations in females during these years.

TABLE X

GONOCOCCAL COMPLEMENT FIXATION TEST

	1937–44	1945–46
Total	388	2,540
Negative ..	38%	64%
Doubtful ..	28%	25%
+	18%	7%
++	16%	4%

Results Obtained with the Gonococcal Complement Fixation Test

In the first instance various methods were tried, including the use of both commercial antigens and those which we prepared ourselves by Price's method (1932 and 1933). It was, however, with a freshly prepared and standardized antigen from 18- to 24-hour cultures made up for each day's work (as recommended by Tulloch, 1923, 1924, 1925) that we obtained results which were to us most convincing. The data over a period of 10 years are given in Table X. From 1937–44 a much smaller number of examinations was done, mostly related to such conditions as suspected gonorrhœal arthritis or salpingitis. In the second period the test was used much more extensively, and as was to be expected the number of positives was considerably lower.

Transport of Material for Culture

There are many practical difficulties which arise in using cultures for this purpose, the most obvious being the rapid transport of material to the laboratory. Results of culture when swabs are sent from a hospital are very different from those obtained from cultures made in venereal disease departments where there are incubators and jars for keeping cultures in air and CO_2 till they are sent to the laboratory. There have been many suggestions for obtaining the secretions and conveying them to the laboratory in good condition. The more popular in the United States have been methods of freezing (Wortman and others, 1941; Sanderson and Allison 1942). The swab, which before use is treated with 15 per cent. glycerine, is put in a tube and the tube immersed in alcohol to which carbon dioxide ice has been added. It is then transferred to a thermos flask to which are added a few lumps of dry ice. Another method recommended is the portable incubator (Harrison, 1937). Both methods have given valuable results, but they are elaborate and include the transmission of heavy apparatus, and dry ice is not in all circumstances easily obtained.

The simplest and most interesting method seems to be one recently described by Stuart (1946), who points out one or two apparently significant theoretical aspects of the subject. For example, the death of the gonococcus by drying is caused by increased rapidity of oxidation: if the gonococci can be kept under conditions of reduction this does not take place. The method which Stuart has finally adopted is to use small screw-capped bottles filled with 0·3 per cent. agar, which gives the most suitable physical conditions; the agar contains one part in 1,000 of thioglycollic acid neutralized with NaOH, and the whole is buffered with 1 per cent. glycerophosphate and 1 part in 10,000 calcium chloride. One part per 500,000 methylene blue is added in order to check the persistence of conditions of reduction. As long as these are satisfactory the agar remains colourless. The swab is

pushed into the bottle, its stem is cut off flush with the rim, and the cap is screwed down. Stuart gives figures showing that by the use of these methods the gonococcus will remain capable of sub-culture up to two, three, or four days, and there is no tendency for the other organisms to grow out: they are at least as much restrained as the gonococcus.

The results of culture and gonococcal complement fixation tests during this period depended mainly on the work of my colleagues Miss Bertha Wheatley and Messrs. K. I. Johnstone, K. Zinnemann, and D. Dolby, to whom I wish to express my indebtedness.

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DISCUSSION ON THE PRECEDING PAPER

DR. G. L. M. MCELLIGOTT (the President) was in favour of cultures as against smears. He had been brought up to the idea that the gonococcus hated the cold, and to know that it could be frozen with impunity was reassuring. He asked what Prof. McLeod thought of portable incubators for use in country clinics where the choice of the best method of transporting the gonococcus from clinic to laboratory was important. Dr. Stuart's work sounded extremely interesting. To a question about how long one kept the gonococcus in the semi-fluid agar, Prof. McLeod replied that it was a matter of preservation: it was kept there until it could be cultivated.

DR. T. E. OSMOND said he had always taught that the gonococcus did not mind the cold; in fact when he had it growing rapidly if he could not sub-culture for a few days he put it in the ice chest during the interval. Prof. McLeod replied that the question of cold might not be so familiar to clinicians as to bacteriologists. Oddly enough the accepted method to-day of preserving all bacteria was to dry them from the frozen state. Dr. Osmond asked whether Prof. McLeod had adopted the method for identifying the gonococcus recommended by Thomson, that is, solubility in weak alkali. In his experience the gonococcus colonies were much more clearly visible on a colourless medium—where one could see the details, the shape, the scalloped edge, and the striations—than on a blood medium. He had used the medium described by Thomson, which contained the usual ingredients plus hydrocele fluid, and also the chocolate medium agar; though the gonococcus might grow as well on the latter, it was much more difficult to see the nature of the colonies. Prof. McLeod said he had not tried Thomson's test. The best colour contrast was largely a question of what one was accustomed to. A chocolate background was quite useful, but he noticed that Reymann on going into this in detail had chosen a chocolate medium reinforced with ascitic fluid. He preferred Dr. Osmond's suggestion of hydrocele fluid. Noguchi, in cultivation of spirochætes, had great difficulty because of the varying qualities of different samples of fluid, and that would probably apply also to the gonococcus. Dr. Osmond asked whether Prof. McLeod considered hæmoglobin an essential or an advantage, and Prof. McLeod replied that the blood pigment appeared to be an important part of the blood in this connexion.

DR. LEES said that formerly he had been sceptical of the superiority of cultures over smears in diagnosing gonorrhœa, but since using the excellent service provided in Leeds he had become convinced that many cases of chronic and latent gonorrhœa, especially in females, could be detected only by efficient cultural examination. It had been a source of surprise and disappointment that so few Army and civilian laboratories could grow gonococci, even in cases where direct smears from the case showed the organisms to be abundant. His former colleague, Dr. Betty Walker, had recently examined a series of 140 consecutive cases in which a diagnosis of gonorrhœa had been made,